A SECOND SERIES OF EXPERIMENTS DEALING WITH THE TRANSMISSION OF GOITRE FROM MAN TO ANIMALS

BY

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OBJECT OF THE RESEARCH

In a former series of experiments, the results of which were communicated to the Royal Society in June (1911), and published in full in the 'Annals of Tropical Medicine and Parasitology' (1911), it was shown that the thyroid glands of goats, fed on water infected with the faeces of goitrous individuals, underwent in 50 per cent. of cases an increase in size and presented the following changes on microscopical examination:—

(1) A marked dilatation of the vesicles with colloid substance, and a thinning of their walls.

(2) A flattening of the epithelial lining of the vesicles.

The object of the present research was:—

(1) To repeat the experiment of feeding goats on a water highly contaminated with the faeces of goitrous individuals.

(2) To ascertain the effect on the thyroid glands of animals of cultures of micro-organisms grown from the faeces of goitrous individuals, when administered by the mouth.

(3) To ascertain the effect on the thyroid glands of animals of such cultures of micro-organisms when administered by the mouth in association with an artificial water containing the carbonates of lime, magnesium and sodium.

(4) To ascertain the effect on the thyroid glands of animals of the above-mentioned carbonates when administered alone.

The experiments were carried out during the months of January to May, 1911. The animals employed were goats and dogs.

EXPERIMENT A.—The administration to goats of water contaminated with the faeces of goitrous individuals.

The method of preparing this water has been detailed in a former communication[2]: a pure water was allowed to percolate through a mixture of sterilized soil and faeces, and collected in a stoppered bottle. Twelve female goats, aged two years, were confined in a pen on a goitre-free soil, and provided with this fouled water as the only drinking water for a period of 107 days (from 20th January to 6th May, 1911) The animals were liberally fed on a ration consisting of one pound of grain and as much lucerne grass as they could eat. All other possible sources of infection were rigidly excluded. It had been observed in my former experiments that the goats employed lost in weight; I, consequently, added the grain to the ration in the case of the animals of the present experiment. At the end of 107 days seven of the twelve goats were taken at random and killed. Their thyroid glands were removed, weighed, and subsequently examined microscopically. Manual examination of the thyroids of these animals during the course of the experiment showed that in about 50 per cent. of cases the glands had increased in size, and were readily palpable. But it was observed that the size of the organ fluctuated considerably; at one examination it was easily felt and its whole outline could be clearly appreciated, while at another it was felt with difficulty.

The following table shows the weights of the thyroid glands as compared with the body weight of the animals:—

No.	Weight of animal	Weight of thyroid	Proportionate weight of thyroid to weight of animal
I	42 lbs.	2·8 gms.	1:7466
II	42 lbs.	3·0 gms.	1:6,999
III	38 lbs.	1·3 gms.	1:14,600
IV	55 lbs.	3·1 gms.	1:8,800
V	45 lbs.	2·5 gms.	1:9,000
VIII	46 lbs.	1·4 gms.	1:16,400
V	28 lbs.	1·7 gms.	1:8,000

The average weight of a normal goat's thyroid gland is, in Gilgit, $\frac{1}{10,000}$th part of the body weight. It will be observed that three of the above animals (Nos. 1, 2 and 7) had thyroid glands considerably larger than normal. The increase in size was not so marked as in the case of my former experiments, where four out of six goats were found to show an increase in size of the organ varying from $\frac{1}{4,500}$th to $\frac{1}{8,000}$th part of the body weight of the animal. This difference may be attributed to the fact that the animals in the former experiments were not so well fed, and, consequently, *lost* in weight, while those of the present series *gained* in weight. It will further be observed that two other goats (Nos. 3 and 6) had thyroid glands considerably less in weight than normal, while these organs in the remaining animals (Nos. 4 and 5) showed no marked deviation from the normal size.

The histological appearances of the glands of these animals varied very considerably:—

Nos. 4 and 5 showed no appreciable deviation from the normal type. A small area of parathyroid-like tissue was seen in the thyroid of No. 5.

Nos. 3 and 6 differed from the normal type in so far that the vesicles were somewhat smaller and the cells lining the vesicles were higher (low columnar) than is the case in the normal gland.

Nos. 1 and 2 were identical in appearance. The vesicles (fig. 3) were on the whole larger, more irregular in shape, and the total amount of colloid was greater than in the case of the normal gland (fig. 1). The epithelium lining the vesicles was somewhat higher (fig. 4) than in the normal gland (fig. 2). The thyroid in these cases did not present the same degree of dilatation and irregularity of the vesicles, nor the marked flattening of the epithelium, which was observed in the case of my former experiments (fig. 5) (1911). Indeed, apart from the increase in size of the organs, it cannot be said that the histological appearances differed to any very marked extent from the normal gland.

No. 7.—The histological appearances of this organ revealed a considerable degree of hyperplasia. The vesicles were small, the amount of colloid comparatively scanty, the lining epithelium was columnar and the vessels of the gland were dilated. The gland was

markedly more cellular than any other in the present experiment (figs. 6 and 7).

The net result of this experiment, therefore, is that of seven thyroid glands examined three (or 42 per cent.) were larger than normal as determined by weight. Of these, two showed in their histological appearances no very marked variation from the normal type, while the third showed a considerable degree of hyperplasia. Two other glands were in all respects normal, while the remaining two were considerably smaller than normal, and showed a more cellular structure and a higher type of epithelium lining the vesicles than is normal.

EXPERIMENT B.—The administration to goats of cultures of bacteria grown from the faeces of goitrous individuals:

Four female goats, aged two years, were housed and fed in a manner identical with those in Experiment A. They were provided with a drinking water of known purity, which, as an additional precaution, was boiled. The animals were given cultures of bacteria, grown from the faeces of goitrous individuals The experiments lasted 108 days (20th January to 7th May, 1911). During the first month the animals were given, on alternate days, 48-hour cultures on Musgrave's agar of such organisms as had grown on this medium. At this stage of the experiment no attempt was made to purify the growth. Musgrave's agar tubes were inoculated direct from the faeces, incubated, and sub-cultures from the resultant growth were made on Musgrave's agar. After forty-eight hours' incubation an emulsion of the bacteria present was made in distilled water, mixed with milk, and introduced into the stomach of the animal through a funnel. The contents of one tube of a 48-hour culture was given to each animal every other day. The organisms present in such a culture were mainly of two classes: (1) Organisms of the coli group, and (2) a spore-bearing organism, the characters of which will be subsequently described. At the end of the second month a pure culture of the spore-bearing organism referred to was obtained from the faeces of a horse which was suffering from recent goitre, and from the 61st day of the experiment onward this organism alone was given—one tube of a 48-hour growth to each animal on alternate days. The animals were all killed on the 7th May, their thyroid glands removed and subsequently examined microscopically. The

following table shows the weight of the thyroid gland in each case relative to the body weight of the animal:—

No.	Weight of animal on 20th Jan., in lbs.	Weight on 7th May, in lbs.	Increase in weight, in lbs.	Weight of thyroid	Proportionate weight of thyroid to body weight of the animal
I	42	54	12	1·0 gm.	1:27,000
II	48	65	17	2·3 gms.	1:13,000
III	37	40	3	2·0 gms.	1:10,000
IV	32	41	9	1·6 gms.	1:12,000

It will be observed from the table:—

(1) That all the animals increased in weight. In case No. 2 the increase in weight is very marked. In case No. 3, in which the thyroid is of normal weight, the animal increased only three pounds in weight.

(2) That in three cases out of four the thyroid gland was considerably smaller than normal.

There was, therefore, in these animals no evidence of any enlargement of the thyroid gland (goitre). But well-marked histological changes were observed in all animals in this experiment in which the thyroid showed any marked deviation from the normal weight.

No. 1.—The animal increased 12 lbs. in weight. The thyroid gland was almost three times smaller than normal. The following changes were observed on microscopical examination: the vesicles were round or oval, lined with cubical or low columnar epithelium; the colloid was small in amount, areas of parathyroid-like tissue were seen, wholly cellular, showing an absence of colloid, and merging into the vesicular structure of the rest of the gland; this cellular structure was observed to form about one-half of the total area of the section; the capillaries and vessels of the stroma were dilated; there was an increase of the connective tissue stroma of the organ, especially around the blood vessels, the walls of which appeared somewhat thickened.

No. 2.—The animal increased 17 lbs. in weight. The thyroid gland was smaller than normal. The following changes were observed on microscopical examination: vesicles were almost wholly absent or were filled with round, imperfectly-staining cells and cellular débris. Where vesicles were seen they were lined with irregular, high columnar epithelium, the lining being often incomplete in parts. Stainable colloid was wholly absent. The stroma was increased markedly in amount and formed a network, the meshes of which were filled with round cells, many of which stained imperfectly, and cellular débris. The capillaries of the organ were not noticeably altered. The large central artery appeared dilated, though its walls were not thickened (figs. 8 and 9). The appearance of this organ and the great increase in weight of the animal are suggestive of a commencing myxoedema.

No. 3.—The animal increased 3 lbs. in weight during the experiment. The thyroid gland was of normal weight. The histological features of the organ were normal. There was a small circumscribed area of parathyroid-like tissue in the centre of the gland.

No. 4.—The animal increased 9 lbs. in weight during the course of experiment. Microscopical examination of the thyroid gland showed it to be rich in colloid, the vesicles rather larger and more irregular than normal. A small cyst was present. No other abnormalities were noted.

The net result of this experiment, therefore, is that of four goats three had thyroid glands smaller than normal, in two of which histological changes were marked. These changes were in one case those of an active hyperplasia, while in the other they amounted to a fibrosis and cellular degeneration of the organ. The fourth gland was in all respects normal.

Experiment C.—The administration to goats of cultures of bacteria, grown from the faeces of goitrous individuals, together with a known quantity of the carbonates of lime, magnesium and sodium.

The conditions of this experiment were identical with those of Experiment B. The same cultures were employed and administered for the same length of time and in the same way. But one hour previous to the administration of the cultures the four female goats employed in the experiment were given a solution of 5 grains each of

the carbonates of lime, magnesium, and sodium. My object in giving the animals these salts was to ascertain whether they exerted any influence in favouring the development of a goitre in the animals to which the bacterial cultures were being given. In certain localities where the drinking water contains large quantities of lime and magnesium, individual goitres are, as a rule, larger than in other localities where the water is less hard, and though these metals are now known not to cause the disease, yet it is possible they may exert an influence of a secondary importance in the production of the malady. In the present experiment it was thought that the administration of these carbonates might, by increasing the alkalinity of the intestinal contents, favour the development of the bacteria administered by the mouth, these bacteria having been cultivated on an alkaline medium. The experiment was carried out concurrently with the previous one, and lasted the same length of time.

Three of the four goats were killed, their thyroid glands removed and weighed, and subsequently examined microscopically. The following table shows in each case the weight of the thyroid gland relative to the body weight of the animal:—

No.	Weight of animal on 20th Jan., in lbs.	Weight of animal on 7th May, in lbs.	Increase in weight in lbs.	Weight of thyroid	Proportionate weight of thyroid to body weight of the animal
I	44½	55	10½	1·5 gms.	1:18,200
II	41	45	14	1·45 gms.	1:16,000
III	37½	45	7½	2·1 gms.	1:10,630

The histological appearances of the glands of these animals were as follows:—

No. 1.—There was an increase in the connective tissue stroma; colloid was scanty; vesicles were rounded or oval, and either filled with cells or lined with a high columnar epithelium. The vessels of the stroma were not noticeably hypertrophied, though some were dilated. These features indicated a well-marked hyperplasia (figs. 10 and 11).

No. 2.—The microscopical appearances were the same as in the

previous case, but the vesicles were larger, and the cells lining them of a lower columnar type, while the colloid was rather more plentiful.

No. 3.—The gland showed little deviation from the normal type. The colloid was plentiful. The vesicles were here and there lined with a low columnar epithelium. An area of the parathyroid-like tissue was present. Serial sections in this, as well as in other cases, showed that the cellular parathyroid-like area gradualiy merged into the vesicular structure of thyroid tissue (fig. 12), from which it appeared to differ in no essential, except in the absence of formed vesicles and colloid.

The net result of this experiment, therefore, is that of three goats two had thyroid glands which showed marked degrees of hyperplasia, while the glands were considerably smaller than normal All these animals increased considerably in weight under the conditions of the experiment.

EXPERIMENT D.—The administration to goats of the carbonates of calcium, magnesium and sodium.

In this experiment four female goats of the same age as those in the preceding experiments were employed. The conditions of the experiment were in all respects identical with those of B and C, except that in this case the animals were given only the carbonates of magnesium, calcium, and sodium in doses of five grains of each of these salts every other day. The experiment was carried out concurrently with the preceding one, and was intended mainly as a control to it. On the expiration of 107 days two of the four goats were taken at random and killed, their thyroids removed, weighed, and subsequently examined microscopically. The following table shows in each case the weight of the thyroid gland relative to the body weight of the animal:—

No.	Weight of animal on 20th Jan., in lbs.	Weight of animal on 7th May, in lbs.	Increase or decrease in in weight in lbs.	Weight of thyroid in gms.	Proportionate weight of thyroid to body weight of animal
I	27½	25	— 2½	1·5 gms.	1:8,400
II	37½	43	+ 5½	2·2 gms.	1:9,700

The histological appearances of the thyroid glands of these animals were as follows:—

No. 1.—No deviation from normal could be observed.

No. 2.—In this case the colloid was comparatively scanty, the vesicles were small or oval, and lined with low columnar epithelium. The stroma around the vessels was increased, though not markedly so, around individual vesicles, and stretched into the gland, giving it under a low power a lobated appearance. The vessels were not noticeably dilated or hypertrophied.

The net result of this experiment, therefore, is that one of two goats showed a slight degree of hyperplasia of the thyroid gland.

SUMMARY OF RESULTS

If the results of these four experiments are compared, several broad differences will be noted:—

(1) In those animals which drank only highly faecal polluted water for over three months there was a tendency on the part of the thyroid gland to be larger than normal (3 cases out of 7).

(2) In those animals which were fed on cultures of bacteria from the intestines of goitrous individuals there was a tendency on the part of the thyroid gland to be smaller than normal, and this tendency appears to be well marked (5 cases out of 7). The diminution in size of the thyroid of these animals appears also to be associated with an increase in their body weight.

(3) In those animals which drank a highly faecal polluted water the histological appearances of the gland either differed in no essential from normal, or there was evidence of an increase in size of the vesicles, of irregularity in their shape, of a higher type of epithelium lining the vesicle, and of a total increase in the amount of colloid present.

(4) In those animals which were fed on cultures of bacteria from the intestines of goitrous individuals, a marked tendency to hyperplasia was observed. The cells lining the vesicles were in a large proportion of the cases columnar in type, colloid was scanty, and there was evidence of an increase in the connective tissue stroma of the organ. In one case the stroma was so markedly increased, and the cells so altered as to give rise to the suggestion of commencing myxoedema.

(5) A slight hyperplasia was also observed in one of two goats to which only carbonates of magnesium, lime and sodium had been given.

It appears, therefore, that a considerable hyperplasia of the thyroid gland may occur under various conditions, as it is present in one or more cases in each of the foregoing experiments. But so marked are the histological changes in some of the thyroid glands of the goats of experiments B and C, and so striking is the contrast between them and the glands of normal animals, and of the goats of the other experiments, A and D, that one is led to attribute these changes to the action of the bacteria administered. The cases are, however, too few to admit of more than this general conclusion being drawn; and this conclusion must be subjected to the test of further experiment on a much larger number of animals than were employed in the present instance.

The results of feeding goats on faecal polluted water was in the present series not so marked as in my former experiment. But here also three goats out of seven showed an enlargement of the thyroid gland, as determined by weight. The structure of these glands, however, did not show the same degree of dilatation and distension of the vesicles with colloid, nor was the thinning and irregularity of the walls of the vesicles so marked, or the epithelial lining so flattened as in the thyroid glands of the goats of my first series of experiments. The results, nevertheless, are on the whole similar to those obtained in my former experiments.[1,2]

I have alluded to a spore-bearing bacillus, which was isolated in pure cultures from the faeces of a goitrous horse, and which was constantly present in the cultures from the faeces of goitrous individuals, as having been employed in experiments B and C. I am indebted to the Director of the Pasteur Institute of Kasauli and to the Assistant Director for the following description of this organism. It is a rod-shaped bacillus, varying from 2 to 4 μ in size, which does not retain the stain by Gram's method, but stains well with Carbo-Fuchsin, Leishman's, and other stains. The bacilli vary in size and thickness, and some of them contain a lighter unstained area, situated usually at the centre, but sometimes towards the periphery of the organism. The growth on Musgrave's agar shows, in addition to the bacilli, numerous round unstained bodies, which are seen tc be

spores when special staining methods are employed. In some of the bacilli spore formation is also seen. The organism is very actively motile in young cultures. Plate cultures on agar and Musgrave's medium appear as small, round and opaque white colonies. The deeper colonies are irregular, with crenated margins, and are bluish white in colour. The growth is more profuse on alkaline (÷ 1) agar than on Musgrave's medium. On agar slopes (÷ 1) a profuse opaque growth, white lead in colour when viewed from the surface, and faintly brown in colour with transmitted light, is seen after twenty-four hours.

The organism ferments glucose, laevulose, mannose, and galactose, but not dextrose, mannite, lactose or maltose. It produces no curdling or acid in litmus milk, and grows profusely in broth, forming a white scum on the surface. It does not liquefy gelatine in stab cultures; in this medium it forms a button-like growth into the medium, with both superficial and deep gas production. On potato there is a profuse brownish growth. The organism is not killed by 0·5 per cent. carbolic acid for twenty-four hours in an incubator, nor by 60° C. for half an hour. It is not destroyed by boiling at 94° C. for five minutes. Half to one c.c. of a living culture injected into guinea-pigs produced no immediate ill effects. Larger quantities of the living culture injected into dogs, kids, and goats gave a similar result.

A young dog, weighing 9 lbs. 4 ozs., was given nine Musgrave's agar tubes of a forty-eight hour culture of this organism. Two hours later the dog was observed to be making violent efforts to vomit, but without result. About six hours later the animal became stiff and unsteady on his limbs. On the following day he was observed to have developed pronounced clonic spasms of the muscles of the limbs and tail. He was then unable to stand, and when propped up his legs gave way under him. These symptoms persisted, the animal lost consciousness, a blood-stained discharge from the mouth, nose, and anus appeared on the fourth day, and the animal died on the ninth day of the experiment. The thyroid lobes were removed, with two enlarged lymph glands in their vicinity. Two agar (÷ 1) tubes were inoculated with the blood-stained fluid which escaped from the cut surface of the lymph glands. After twenty-four hours' incubation several colonies of the spore-bearing organism above described were

present in one of the tubes. The other tube remained sterile. The thyroid gland of the animal was about one-third of the size of another dog, which weighed 10 lbs. 6 ozs., and which was killed for purposes of comparison. The organ on microscopical examination was found to be almost wholly cellular, with an occasional vesicle of normal appearance scattered here and there through the section. The connective tissue stroma appeared to be increased, and formed a well-marked network, the meshes of which were filled with round cells, and with no attempt at the formation of vesicles. Colloid was wholly absent except in the few scattered vesicles. The central artery of the gland appeared considerably thickened. The appearances seen are shown in fig. 13, magnified about 500 diameters. Fig. 14 shows the thyroid of the 'bazaar' dog which was killed for purposes of comparison. The magnification is in both cases the same.

Two other dogs were subsequently given the same number of tubes of a forty-eight hour culture of this organism on Musgrave's medium, but the animals remained to all appearances quite healthy. They were, unfortunately, not killed.

If the micro-photograph of this animal's thyroid is compared with that of goat No. 2 B (figs. 8 and 9), which was fed on cultures of this organism for a period of two months, a striking resemblance will be observed in the two cases. There is the same increase of fibrous tissue, the same cellular structure of the gland, broken up into columns of rounded cells by the network of stroma, the same absence of colloid, complete in the case of the goat, partial and limited to a few scattered vesicles in the case of the dog. Fig. 13 also resembles very closely that of a dog's thyroid figured by Mr. Edmunds in his work on: 'The Pathology and Diseases of the Thyroid Gland'[3] (fig. 19, p. 36); this figure shows the changes in a dog's thyroid 'four days after partial removal.' Mr. Edmunds, in speaking of the changes which take place in the thyroid as a result of removal of the parathyroids, refers to this particular case as one in which, so far from the thyroid increasing in size, it appeared to have been smaller than normal. The small size of this dog's thyroid and of the goats' thyroids in my experiments B and C is very striking

That the experiments detailed in this paper have yielded striking results there is no doubt, but I do not at this stage of my observations propose to do more than record them. The number of animals

employed was too small to permit of definite conclusions being drawn, but the results here recorded are sufficiently marked to justify the belief that experiments conducted on a larger scale, and on the same lines, would yield valuable information. In connection with these experiments, I would draw attention to a paper which I read before the Royal Society of Medicine on the subject of the 'Vaccine Treatment of Goitre,' and in which I detail the results of treatment of incipient goitre by vaccines prepared from coliform bacilli, staphylococcus, and the spore-bearing organism described in this paper.

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2. ——— ,, Ann. Trop. Med. & Parasitol., Aug. 1, V, 2, pp. 187-198.
3. Edmunds, W. The Pathology and Diseases of the Thyroid Gland.

DESCRIPTION OF PLATES

Plate XX

Fig. 1. Thyroid gland of normal control goat. × 100.

Fig. 2. Thyroid gland of normal control goat. × 500.

Fig. 3. Thyroid gland of goat No. 2 (Experiment A). The vesicles are more irregular in shape and the total amount of colloid is greater than in the normal gland. × 100.

Fig. 4. Thyroid gland of the same animal magnified 500 times. The micro-photograph shows the more irregular shape of the vesicles and the higher type of the epithelium lining the vesicles than in the case of the normal gland.

Fig. 5. Thyroid gland of goat, showing dilatation and irregularity in shape of vesicles, thinning of vesicular walls, and increase in the amount of colloid. From a case of artificially-produced thyroid enlargement in a goat (1911). × 700.

Fig. 6. Thyroid gland of goat No. 7 (Experiment A), showing scanty amount of colloid, columnar epithelium lining the vesicles, and dilatation of vessels. × 100.

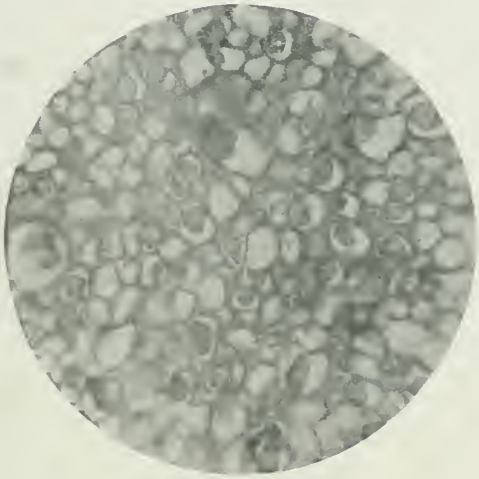

FIG. 1 × 100.

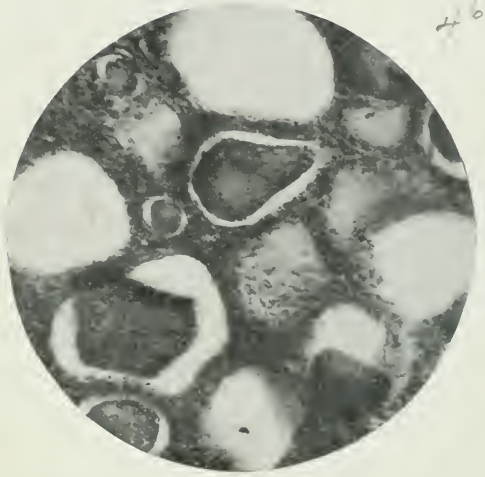

FIG. 2 × 500.

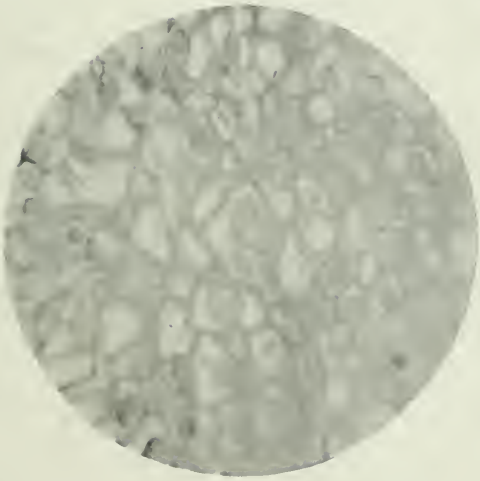

FIG. 3 × 100.

FIG. 4 × 500.

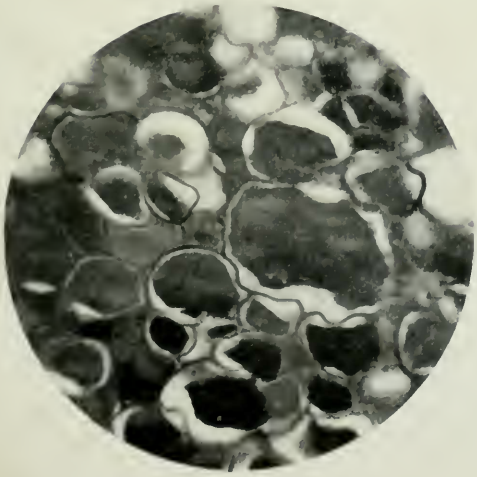

FIG. 5 × 100.

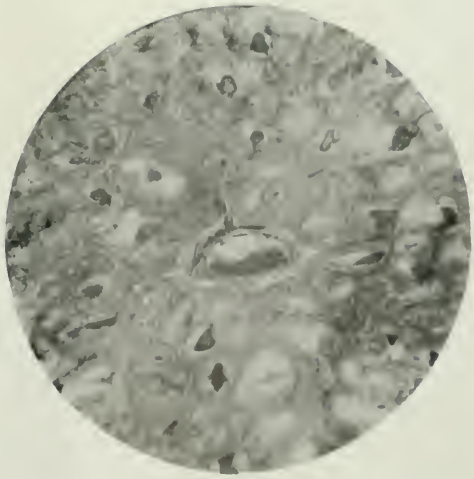

FIG. 6 × 100.

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Plate XXI

Fig. 7. Thyroid gland of the same animal magnified 500 times; showing the columnar epithelial lining of the vesicles, and the dilated vessels in the lower part of the field.

Fig. 8. Thyroid gland of goat No. 2 (Experiment B), showing almost complete absence of vesicles and colloid. × 100.

Fig. 9. Thyroid gland of the same animal magnified 500 times, showing the increase of the fibrous stroma and the masses of cells filling the meshes of the stroma, also the complete absence of colloid.

Fig. 10. Thyroid gland of goat No. 1 (Experiment C), showing vesicles lined with high columnar epithelium, and the scanty amount of colloid. × 100.

Fig. 11. Thyroid gland of the same animal magnified 500 times, showing the high columnar epithelium lining the vesicles.

Fig. 12. Thyroid gland of goat No. 3 (Experiment C), showing area of parathyroid-like tissue in lower left quadrant of field. × 100.

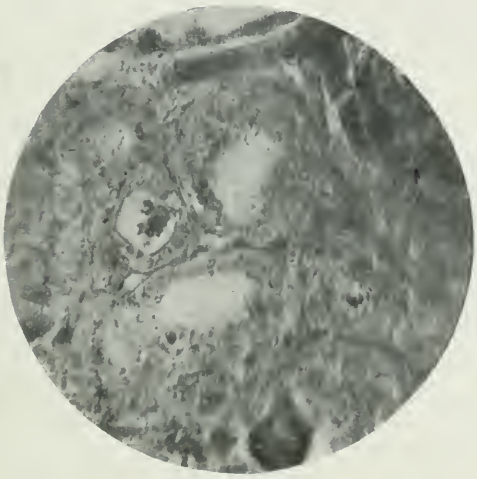

FIG. 7 × 500.

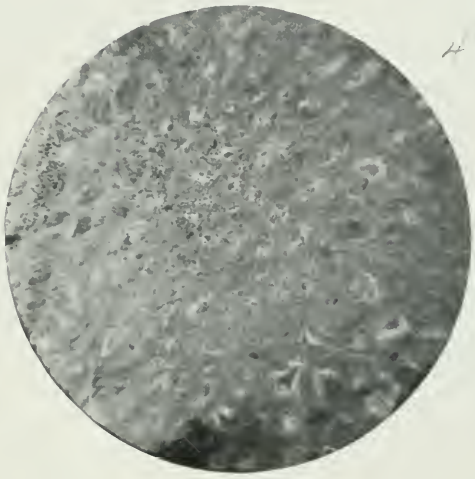

FIG. 8 × 100.

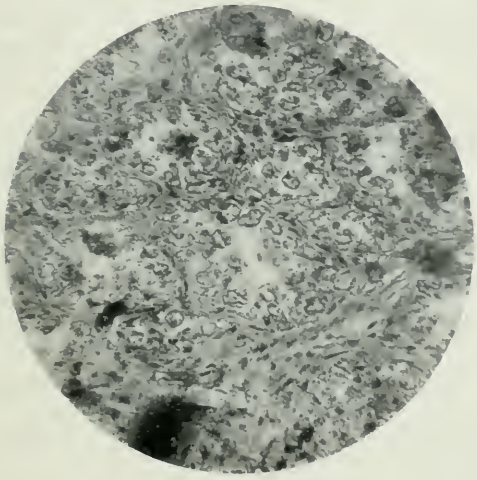

FIG. 9 × 100.

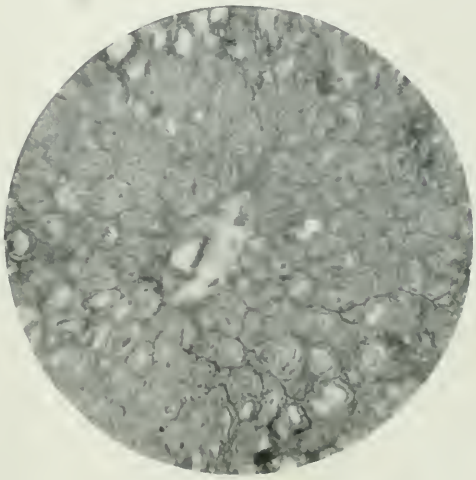

FIG. 10 × 100.

FIG. 11 × 500.

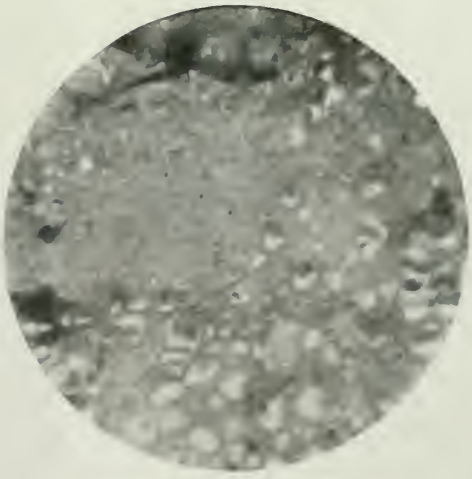

FIG. 12 × 100.

P. P. Press, Imp.

Plate XXII

Fig. 13 Thyroid gland of a dog, showing the cellular structure of the organ, and the absence of vesicles and of colloid. × 500.

Fig. 14. Thyroid gland of normal 'bazaar' dog, showing the normal appearance of the thyroid gland of a dog. × 500.

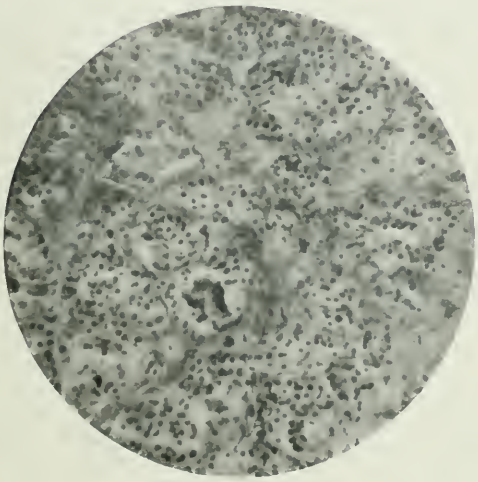

FIG. 13 × 500.

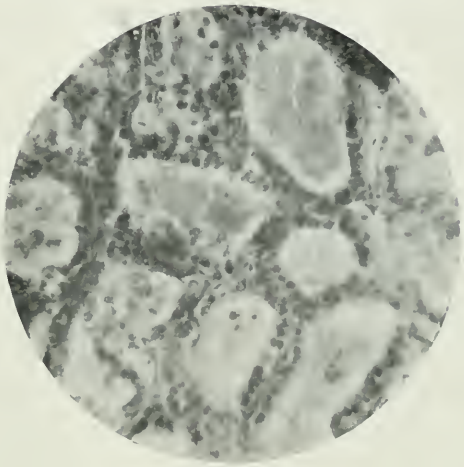

FIG. 14 × 500.

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